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Pinkment: a synthetic platform for the development of fluorescent probes for diagnostic and theranostic applications†

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Reaction-based fluorescent-probes have proven successful for the visualisation of biological species in various cellular processes. Unfortunately, in order to tailor the design of a fluorescent probe to a specific application (*i.e.* organelle targeting, material and theranostic applications) often requires extensive synthetic efforts and the synthetic screening of a range of fluorophores to match the required synthetic needs. In this work, we have identified Pinkment-OH as a unique “plug-and-play” synthetic platform that can be used to develop a range of ONOO⁻ responsive fluorescent probes for a variety of applications. These include theranostic-based applications and potential material-based/bioconjugation applications. The as prepared probes displayed an excellent sensitivity and selectivity for ONOO⁻ over other ROS. *In vitro* studies using HeLa cells and RAW 264.7 macrophages demonstrated their ability to detect exogenously and endogenously produced ONOO⁻. Evaluation in an LPS-induced inflammation mouse model illustrated the ability to monitor ONOO⁻ production in acute inflammation. Lastly, theranostic-based probes enabled the simultaneous evaluation of indomethacin-based therapeutic effects combined with the visualisation of an inflammation biomarker in RAW 264.7 cells.

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Introduction

There is a growing need for new and effective diagnostic tools that can evaluate biomarkers involved in inflammatory based diseases.^{1–6} Inflammation is the innate defence mechanism of the body that recognises damaged cells, pathogens and

infections. The inflammatory response often results in the generation of reactive oxygen species/reactive nitrogen species (ROS/RNS), which are involved in the functional regulation of M1 and M2 macrophages.^{7,8} The M1 pro-inflammatory phenotype is induced by lipopolysaccharide (LPS), which triggers the generation of ROS from NADPH using NADPH oxidase (NOX).⁹ This production of ROS regulates an array of cellular events including the activation of the nuclear factor kappa-B (NF-κB), the production of cytokines and cell survival whereas, high levels of ROS are associated with programmed cell death, *i.e.* apoptosis.^{7,10–14} The high sensitivity and high spatial and temporal resolution of fluorescent probes allow us to visualise these key cellular events. Our group and others have focused on the fluorescence-based detection of ROS/RNS such as ONOO⁻, H₂O₂ and HOCl.^{1,15–21} To achieve the selective detection of a particular ROS requires the careful consideration of both fluorophore and reactive motif. In this regard, resorufin is a particularly attractive fluorophore due to its red shifted fluorescence and easy to functionalise scaffold. Pioneering work led by Chang *et al.* developed peroxyresorufin-1 (PR1) for H₂O₂ detection whereby resorufin is masked with boronic esters.^{22,23} Boronic esters have been identified as a relevant sensing group for both H₂O₂ and ONOO⁻ detection. However, in an environment with both species present, boronic esters preferentially react with ONOO⁻ due to the inherent faster reactivity of ONOO⁻ in comparison to H₂O₂.²⁴ Previously, we have

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Fig. 1 (A) Chemical structure of the resorufin-based probes for the sensing of ONOO^- , including the previously reported **Pinkment-OH** “plug and play” scaffold and dual analyte probes **Pinkment-OTBS** and **Pinkment-OAc**.¹⁰ (B) Unexpected fluorescence turn on response of **1** in the presence of ONOO^- .

demonstrated PR1's ability to preferentially detect ONOO^- over H_2O_2 *in vitro*.²⁵ Consequently, we decided to investigate functionalized synthetic derivatives of PR1.²⁶ This led to the development of **Pinkment-OH** for the design of dual analyte AND-logic probes, **Pinkment-OTBS** (ONOO^- “AND” fluoride) and **Pinkment-OAc** (H_2O_2 “AND” esterase) using **Pinkment-OH** (Fig. 1) as a synthetic starting point. These results revealed the potential of **Pinkment-OH** to be used as a synthetic platform for the development of ONOO^- selective fluorescence probes with additional sensing, targeting or drug units. Here, we have serendipitously discovered that the benzyl unit of our ROS **Pinkment** fluorescent probe can be functionalized with a functional unit of choice without compromising ROS selectivity. As a result, **Pinkment-OH** was successfully shown as a synthetic platform to develop ONOO^- selective fluorescent probes with additional functional units (Fig. 1).

Results and discussion

Initially, our focus was on continuing the development of “AND”-based logic-gates for biological application.^{1,17,26} This led to the elaboration of probe **1**, which was accessed in a simple three step synthesis (Scheme S1†). Unexpectedly, we discovered that **1** “turned on” in the sole presence of ONOO^- (Fig. 1B and S1†). This led to the development of **Pinkment** probes **2** and **3** to further confirm this observation. These probes were accessible from the synthetic platform **Pinkment-OH**, whose 6-step synthesis has been previously reported by our group.²⁶ Nucleophilic substitution by **Pinkment-OH** using 1-bromopropane and pentanoyl chloride respectively gave **2** and **3** in moderate yields: 50% and 51% respectively (Scheme S2 and S3†). Probes **2** and **3** showed good selectivity towards ONOO^- over other ROS species (Fig. S2–S8†). Surprisingly, the probes demonstrated a high sensitivity towards ONOO^- requiring concentrations in the low micromolar range. Both **2** and **3** displayed increased solubility in comparison to **1**. We decided to further explore this

unexpected result by introducing a terminal nitrile group. Probe **4** was accessible in a facile three-step synthesis (Scheme S4†) in the same manner as **1**. Again, good selectivity and sensitivity for ONOO^- was observed (Fig. S2–S8†). From these results, we realized that the **Pinkment** benzyl unit can be functionalized with any unit of choice without compromising the ROS selectivity. Thus, we rationalized that **Pinkment-OH** offers a unique platform for the design of ONOO^- selective fluorescence based probes that can be tailored towards a range of applications.^{27–29} This led to the development of alkyne-based **Pinkment** probes **5** and **6** that have potential to be used in “click” chemistry.²⁹ These probes were accessed from **Pinkment-OH** and prepared in moderate yields: 48% and 47% for **5**, and **6** respectively (Scheme S5 and S6†). Fluorescence studies of **5** and **6** established good sensitivity and selectivity towards ONOO^- over other ROS (Fig. S2–S8†).

We then turned our attention to assessing the imaging capacity of probes **2** and **3** and the potential “click” based probes **5** and **6** in cells and live animals. To demonstrate their suitability as imaging tools, all four probes were evaluated for cellular toxicity in murine RAW 264.7 macrophages using a MTS cell proliferation assay. Probes **2**, **3**, **5** and **6** were incubated at different concentrations ranging from 5 to 40 μM for 24 h (Fig. S9†). Probes **2**, **5** and **6** were found to be non-toxic. In contrast, probe **3** decreased the cell viability of RAW 264.7 macrophages by 40% at a concentration of 40 μM compared to control conditions. As a result, **3** was not taken forward for further cell studies since high concentrations of the probe are required for *in vivo* studies.

Probes **2**, **5** and **6** were shown to be non-toxic, and were evaluated with exogenous ONOO^- , using SIN-1 (500 μM) in RAW 264.7 macrophages (Fig. 2A and S10†). Each probe alone demonstrated minimal fluorescence in cells, the addition of SIN-1 led to a significant enhancement in intracellular fluorescence at a wavelength corresponding to the dye, resorufin, therefore, suggesting the intracellular reaction of the probe with



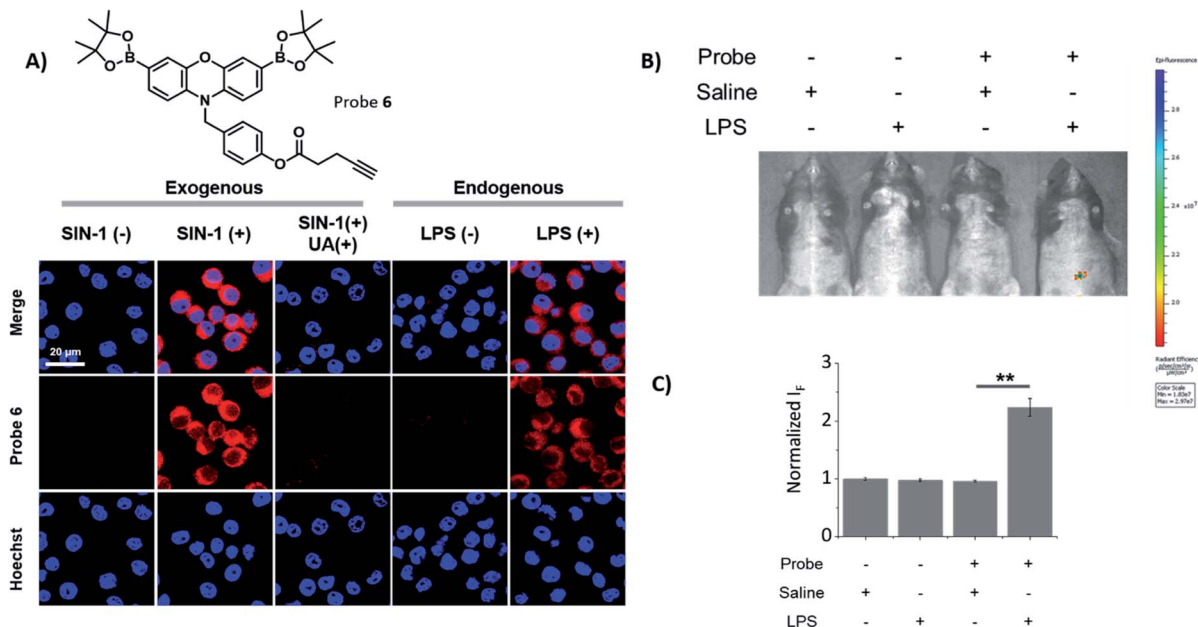


Fig. 2 (A) Confocal imaging of RAW 264.7 macrophages treated with probe 6 (20 μM , 30 min) in the presence and absence of SIN-1 (500 μM , 30 min) and uric acid (100 μM , 2 h) or LPS (1 $\mu\text{g mL}^{-1}$, 24 h) as indicated. Fluorescence data was collected using $\lambda_{\text{ex}} = 559 \text{ nm}$ and $\lambda_{\text{em}} = 580\text{--}650 \text{ nm}$, respectively. The cell nuclei was stained using Hoechst 33342 and fluorescence collected at $\lambda_{\text{ex}} = 405 \text{ nm}$ and $\lambda_{\text{em}} = 450\text{--}480 \text{ nm}$. Scale bar = 20 μm . $N = 3$. (B) Intraperitoneal injection of male C57BL/6J mice with probe 6 (200 μM) or saline in the absence and presence of LPS (2 mg mL^{-1} in saline) with $\lambda_{\text{ex}} = 535 \text{ nm}$ and $\lambda_{\text{em}} = 600 \text{ nm}$. $N = 3$. (C) Quantification of (B) C57BL/6J male mice treated with probe 6 (200 μM) or saline in the absence and presence of LPS (2 mg mL^{-1} in saline) with $\lambda_{\text{ex}} = 550 \text{ nm}$ and $\lambda_{\text{em}} = 580\text{--}620 \text{ nm}$. Error bars represent s. d. with $**p \leq 0.01$. $N = 3$. Normalised fluorescence intensities were calculated using the saline solution fluorescence intensities.

ONOO^- and their suitability for use as fluorescence-based probes. The SIN-1 generated fluorescence signal was then evaluated with the ONOO^- scavenger, uric acid.³⁰ As expected, uric acid attenuated the fluorescent increase that was induced by SIN-1 for all probes, thus confirming the ONOO^- mediated increase in fluorescence intensity. Next, we evaluated the capability of 2, 5 and 6 to detect endogenous ONOO^- in LPS primed RAW 264.7 macrophages. All three probes were shown to detect endogenous ONOO^- in LPS primed RAW 264.7 macrophages (Fig. 2A and S11[†]), confirming their promise for the imaging of LPS-induced inflammatory responses. In addition, HeLa and A549 cell lines treated with or without SIN-1 were used to illustrate the versatility of the **Pinkment** probes (Fig. S12 and S13[†]).

Encouraged by these cell imaging results, we used a known LPS-induced inflammation mouse model³¹ for the *in vivo* detection of ONOO^- (Fig. 2B). The injection of LPS (2 mg mL^{-1} in saline) to the abdominal region of mice followed by the injection of 6 (200 μM) led to its fluorescence activation. The quantified fluorescence intensity in the probe(+)/LPS(+) group was significantly larger than that in the probe(+)/LPS(-) group (Fig. 1C), demonstrating the potential of using 6 for the monitoring of ONOO^- *in situ* during acute inflammation.

In order to follow our current interest in theranostics,³² we then turned our attention towards the potential of **Pinkment-OH** for the design of fluorescence-based drug releasing probes. Therefore, we used the drugs chlorambucil and indomethacin to afford two distinct theranostic probes 7 and 8, respectively

(Fig. 1A). Chlorambucil is used to treat chronic lymphatic leukemia³³ and indomethacin is used as a non-steroidal anti-inflammatory drug (NSAID).^{34,35} Both 7 and 8 were easily accessible from **Pinkment-OH** (Scheme S7 and S8[†]).

Mass spectrometry confirmed and validated the simultaneous release of each drug and fluorescent resorufin dye (Fig. S14 and S15[†]). Therefore, the enhancement in the fluorescence intensity over time indicates the release of each drug. As such, time-dependent fluorescence experiments with 7 and 8 in the presence of ONOO^- were performed to illustrate the time dependence of the drug release. These experiments revealed a maximum fluorescence response after $\sim 10 \text{ min}$ (Fig. S16[†]).

Fluorescence studies were carried out including ROS selectivity, H_2O_2 titration and ONOO^- titration studies (Fig. S17–S21[†]) and demonstrated high sensitivity towards these inflammation-based biomarkers. Following these initial studies, we evaluated both 7 and 8 in RAW 264.7 macrophages towards exogenous ONOO^- detection (Fig. S22[†]). The presence of SIN-1 significantly enhanced the intracellular fluorescence of 7 and 8, confirming the applicability of the probes *in vitro*. Despite 7 displaying significant promise, the creation of an appropriate model system to differentiate between cancerous and healthy cells would require a significant amount of development and as such was beyond the scope of this current research. Therefore, only 8 was further evaluated, since its cellular behaviour was easier to monitor. Endogenous ONOO^- was also detected by 8 in RAW 264.7 macrophages (Fig. 3A). Indomethacin, a NSAID, is an effective and non-selective





Fig. 3 (A) Confocal imaging of RAW 264.7 macrophages treated with LPS ($1 \mu\text{g mL}^{-1}$, 24 h) and then loaded with **8** ($20 \mu\text{M}$, 30 min) as indicated. Fluorescence data was collected using $\lambda_{\text{ex}} = 559 \text{ nm}$ and $\lambda_{\text{em}} = 580\text{--}650 \text{ nm}$, respectively. The cell nuclei were stained using Hoechst 33342 and fluorescence collected at $\lambda_{\text{ex}} = 405 \text{ nm}$ and $\lambda_{\text{em}} = 450\text{--}480 \text{ nm}$. Scale bar = $100 \mu\text{m}$. $N = 3$. (B) Effect of **8** on LPS-induced COX-2 gene expression in RAW 264.7 macrophages. Cells were treated with LPS alone ($1 \mu\text{g mL}^{-1}$) or together with **8** for 24 h. Indomethacin was set as a positive control, and the relative mRNA level of COX-2 gene was normalized by GAPDH ($*p < 0.05$). $N = 4$.

inhibitor of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), of which COX-2 is mainly responsible for the inflammatory response.³⁶ The therapeutic effects on the LPS-induced inflammatory responses in RAW 264.7 macrophages were further investigated using **8**. RAW 264.7 macrophages were treated with LPS and the expression of the pro-inflammatory gene (COX-2) was investigated using qRT-PCR in the presence or absence of **8** (Fig. 3B).³⁷ The mRNA level of COX-2 decreased in the presence of **8** ($50 \mu\text{M}$) in comparison to the LPS-induced group. A similar effect to the LPS-induced group was observed with indomethacin alone. This suggests that **8** can monitor ONOO⁻ production in acute inflammation, and in addition, reduce the inflammatory response by releasing indomethacin.

Conclusions

The ability of the **Pinkment** scaffold to be functionalised with any unit of choice without compromising the overall ROS selectivity, opens up new possibilities for the design of highly specific ONOO⁻ probes that can be used in a variety of applications. In this work, we have successfully illustrated the applicability of **Pinkment**-based probes for diagnostic and theranostics applications. Our probes displayed good selectivity and sensitivity towards ONOO⁻ over a range of other ROS. Cellular studies with the **Pinkment** probes led to the identification of alkyne-functionalised **Pinkment** probe **6** as a suitable candidate for *in vivo* studies using an inflammatory mouse model. These promising results led us to design potential theranostic probes **7** and **8** with candidate **8** displaying promising properties *in vitro*. We believe this work demonstrates **Pinkment-OH** as a useful synthetic platform to enable the rapid

development of a ONOO⁻ fluorescent probe that can be tailored to the needs of the chemical biologist. In particular, the alkyne **Pinkment** probes offer the possibility of attaching any desired unit *via* click chemistry. Therefore, we anticipate that the **Pinkment** scaffold can be further elaborated for the development of dual analyte, organelle targeting and theranostic probes for a range of diagnostic and theranostic applications.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- X. L. Hu, N. Kwon, K. C. Yan, A. C. Sedgwick, G. R. Chen, X. P. He, T. D. James and J. Yoon, *Adv. Funct. Mater.*, 2020, **30**, 1907906.
- D. Wu, A. C. Sedgwick, T. Gunnlaugsson, E. U. Akkaya, J. Yoon and T. D. James, *Chem. Soc. Rev.*, 2017, **46**, 7105–7123.
- S. Erbas-Cakmak, S. Kolemen, A. C. Sedgwick, T. Gunnlaugsson, T. D. James, J. Yoon and E. U. Akkaya, *Chem. Soc. Rev.*, 2018, **47**, 2228–2248.
- A. C. Sedgwick, L. L. Wu, H. H. Han, S. D. Bull, X. P. He, T. D. James, J. L. Sessler, B. Z. Tang, H. Tian and J. Yoon, *Chem. Soc. Rev.*, 2018, **47**, 8842–8880.
- J. Chan, S. C. Dodani and C. J. Chang, *Nat. Chem.*, 2012, **4**, 973–984.
- K. C. Yon, A. C. Sedgwick, Y. Zang, G. R. Chen, X. P. He, J. Li, J. Yoon and T. D. James, *Small Methods*, 2019, **3**, 1900013.
- A. Weidinger and A. V. Kozlov, *Biomolecules*, 2015, **5**, 472–484.
- B. Ghesquiere, B. W. Wong, A. Kuchnio and P. Carmeliet, *Nature*, 2014, **511**, 167–176.
- A. Kumar, S. H. Chen, M. B. Kadiiska, J. S. Hong, J. Zielonka, B. Kalyanaraman and R. P. Mason, *Free Radical Biol. Med.*, 2014, **73**, 51–59.
- B. Halliwell, *Annu. Rev. Nutr.*, 1996, **16**, 33–50.
- H. Wiseman and B. Halliwell, *Biochem. J.*, 1996, **313**, 17–29.
- J. S. Beckman and W. H. Koppenol, *Am. J. Physiol.: Cell Physiol.*, 1996, **271**, C1424–C1437.
- M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur and J. Telser, *Int. J. Biochem. Cell Biol.*, 2007, **39**, 44–84.
- H. Y. Tan, N. Wang, S. Li, M. Hong, X. B. Wang and Y. B. Feng, *Oxid. Med. Cell. Longevity*, 2016, **2016**, 2795090.
- A. C. Sedgwick, R. S. L. Chapman, J. E. Gardiner, L. R. Peacock, G. Kim, J. Yoon, S. D. Bull and T. D. James, *Chem. Commun.*, 2017, **53**, 10441–10443.
- A. C. Sedgwick, H. H. Han, J. E. Gardiner, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2017, **53**, 12822–12825.
- A. C. Sedgwick, H. H. Han, J. E. Gardiner, S. D. Bull, X. P. He and T. D. James, *Chem. Sci.*, 2018, **9**, 3672–3676.
- A. C. Sedgwick, W. T. Dou, J. B. Jiao, L. L. Wu, G. T. Williams, A. T. A. Jenkins, S. D. Bull, J. L. Sessler, X. P. He and T. D. James, *J. Am. Chem. Soc.*, 2018, **140**, 14267–14271.
- L. L. Wu, H. H. Han, L. Y. Liu, J. E. Gardiner, A. C. Sedgwick, C. S. Huang, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2018, **54**, 11336–11339.
- A. C. Sedgwick, J. E. Gardiner, G. Kim, M. Yevglevskis, M. D. Lloyd, A. T. A. Jenkins, S. D. Bull, J. Yoon and T. D. James, *Chem. Commun.*, 2018, **54**, 4786–4789.
- L. L. Wu, A. C. Sedgwick, X. L. Sun, S. D. Bull, X. P. He and T. D. James, *Acc. Chem. Res.*, 2019, **52**, 2582–2597.
- B. C. Dickinson, C. Huynh and C. J. Chang, *J. Am. Chem. Soc.*, 2010, **132**, 5906–5915.
- E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652–16659.
- A. Sikora, J. Zielonka, M. Lopez, J. Joseph and B. Kalyanaraman, *Free Radical Biol. Med.*, 2009, **47**, 1401–1407.
- M. Weber, A. B. Mackenzie, S. D. Bull and T. D. James, *Anal. Chem.*, 2018, **90**, 10621–10627.
- M. L. Odyniec, A. C. Sedgwick, A. H. Swan, M. Weber, T. M. S. Tang, J. E. Gardiner, M. Zhang, Y. B. Jiang, G. Kociok-Kohn, R. B. P. Elmes, S. D. Bull, X. P. He and T. D. James, *Chem. Commun.*, 2018, **54**, 8466–8469.
- A. Marrocchi, A. Facchetti, D. Lanari, S. Santoro and L. Vaccaro, *Chem. Sci.*, 2016, **7**, 6298–6308.
- Y. P. Chen, Y. L. Xianyu, J. Wu, B. F. Yin and X. Y. Jiang, *Theranostics*, 2016, **6**, 969–985.
- H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
- D. C. Hooper, S. Spitsin, R. B. Kean, J. M. Champion, G. M. Dickson, I. Chaudhry and H. Koprowski, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 675–680.
- D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765–769.
- M. L. Odyniec, H. H. Han, J. E. Gardiner, A. C. Sedgwick, X. P. He, S. D. Bull and T. D. James, *Front. Chem.*, 2019, **7**, 775.
- M. J. Reese, D. W. Knapp, K. M. Anderson, J. A. Mund, J. Case, D. R. Jones and R. A. Packer, *PLoS One*, 2018, **13**, e0203517.
- K. Seibert, Y. Zhang, K. Leahy, S. Hauser, J. Masferrer and P. Isakson, in *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury 2, Pts A and B*, ed. K. V. Honn, S. Nigam and L. J. Marnett, 1997, vol. 400, pp. 167–170.
- A. A. Onischuk, T. G. Tolstikova, I. V. Sorokina, N. A. Zhukova, A. M. Baklanov, V. V. Karasev, G. G. Dultseva, V. V. Boldyrev and V. M. Fomin, *J. Aerosol Med. Pulm. Drug Delivery*, 2008, **21**, 231–243.
- C. Chen, *Nat. Chem. Biol.*, 2010, **6**, 401–402.
- M. Kawashima, N. Ogura, M. Akutsu, K. Ito and T. Kondoh, *J. Oral Pathol. Med.*, 2013, **42**, 499–506.

